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(54) UBE2G1 GENE-DEFECTIVE NONHUMAN ANIMAL

(57)Abstract:

PROBLEM TO BE SOLVED: To reveal the physiological roll of an ubiquitin-binding enzyme(UBE2G 1), especially the action thereof to the skeletal muscle, and to provide a model animal, i.e., UBE2G1 gene-defective nonhuman animal useful for studying a method for elucidating various kinds

of conditions associated with the enzyme in the skeletal muscle, or for treating the conditions.

SOLUTION: This UBE2G1 gene-defective nonhuman animal includes the UBE2G gene substantially having lost the enzymic ubiquitin-binding activities by the deletion of the whole or a part of the UBE2G1 gene encoding the UBE2G1, or the insertion or the substitution of another gene at an arbitrary site.

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CLAIMS

[Claim(s)]

[Claim 1] The UBE2G1 genetic-defect nonhuman animal which comes to lose ubiquitin joint enzyme activity substantially by having inserted or permuted the gene of others [genes / which carries out the code of ubiquitin joining enzyme UBE2G1 / all or a part of / UBE2G1] by deletion or the arbitration part.

[Claim 2] The nonhuman animal according to claim 1 whose gene which carried out deletion is the 2nd exon of UBE2G1 gene.

[Claim 3] The UBE2G1 genetic-defect nonhuman animal according to claim 1 other genes of whose are neomycin resistance genes.

[Claim 4] The UBE2G1 genetic-defect nonhuman animal according to claim 1 to 3 which is a gay deficit mold or a hetero deficit mold.

[Claim 5] The UBE2G1 genetic-defect nonhuman animal according to claim 1 to 3 which is a gay deficit mold.

[Claim 6] The UBE2G1 genetic-defect nonhuman animal according to claim 5 which is a mesangium hardening model animal.

[Claim 7] The UBE2G1 genetic-defect nonhuman animal according to claim 1 to 6 whose nonhuman animal is a mouse.

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DETAILED DESCRIPTION

[Detailed Description of the Invention]

[0001]

[Industrial Application] This invention relates to the nonhuman mammal which comes to lose ubiquitin joint enzyme activity substantially by suffering a loss in the gene which carries out the code of the ubiquitin joining enzyme.

[0002]

[Description of the Prior Art] Are isolated by the gene engineering technique, and when clarifying the function of cloned various genes In recent years as the analysis of a physiological function within the organism of the cloned gene, or a model system of a gene disease The approach of controlling the manifestation of the genetic trait of the internality in which addition or its living thing has artificially the genetic trait of the foreignness with which that living thing originally is not equipped comes to be developed, various transgenic animals are made, and the experiment and research using this transgenic animal prosper.

[0003] Conventionally, if a transforming gene is injected into an embryonic stem cell (Embryonic stem cell: embryonic stem cell), creation of various gene variation animals is tried using the property which can participate in formation of a germ body and can form a chimera animal (Evans, M.J.and Kaufman, M.H., Nature, 292, 154 (1981)). And the research using production and this using an embryonic stem cell of a genetic defect mouse is progressing quickly after that (503 [Thomas & Capecchi, Cell, 51, and]:(1987) 255 [Bradley, et al., Nature, 309, and]:(1984) Koller, et al., Proc.Natl.Acad.Sci.USA, 86, and 8927 (1989)). Moreover, establishment of an embryonic stem cell strain is needed for production of the above-mentioned gene mutant mouse. the most is established from the mouse of 129 systems (it Robertson(s) and Nature(s) ES-D3 cell; -- Doetschman, J.Embryol.Exp.Molph., 87, and 27:(1981) CCE cell; --) 323 445 : (1986) BL / 6III cells; Ledermann, and Burki, Exp.Cell Res., 197, and 254 (1986). As a gene

expression insufficient animal created using such an embryonic stem cell A HPRT genetic defect mouse (292 [Hooper, et al., Nature, 326, and]:(1987) Knehn, et al., Nature, 326, and 295 (1987)), p53 deletion mouse which carried out deletion of the p53 which is one of the antioncogenes (Donehower, et al., Nature, 356, and 215 (1992)), A beta 2-microglobulin gene mutant mouse (Zijlstra, et al., Nature, 344, and 742 (1990)), The RAG-2 (V(D) J recombination activation gene) mutant mouse of an immunopathy model mouse (Sinkai, et al., Cell, 68, 855, etc. are reported (1992).) In recent years, an angiotensinogen genetic defect animal (JP,8-131021,A), The stromelysin-1 deficit transgenic mouse in which the stromelysin which is matrix METARO secreted by fibroblast and chondrocyte and an enzyme kind of proteinase carried out deletion (Patent Publication Heisei No. 507925 [eight to] official report), An immune system disease and the TREB5 genetic-defect mouse permuted by the neomycin resistance gene in a part of TREB5 gene usable as a model animal of an osteodystrophy (JP,8-140527,A), The L-PGDS genetic defect mouse with which Exon V was permuted by the neomycin resistance gene from the exon II of a RIPOKARIN mold prostaglandin D synthetic enzyme gene (JP,11-332417,A), The double knockout gene recombination nonhuman mammal used as the septicemia model permuted with the imprint gene which carries out the code of the imprint gene which carries out the code of Homo sapiens CD 4 so that the manifestation of a mouse of CD4 and CD8 may be lacked, and the allele of a HLA DG seat (JP,9-500025,A), Various nonhuman animals which suffered a loss in the gene originally held by the living thing, or were permuted with other genes, such as a myeloperoxidase gene functional deficit mouse (JP,2000-116280,A), are created. For this contractor of this field, the production approach of the above-mentioned mutant mouse is the usual technique, and is already this alteration technique (the volume for Tetsuo Noda, the experimental medicine, a special number, 14 (20) (1996), Yodosha). It is indicated.

[0004] It is very important that protein is maintained by the proper condition also quantitatively and qualitatively on the other hand, when controlling many functions of a cell. A ubiquitin system is a system which ubiquitin-izes a protein matrix selectively and leads it to proteasome decomposition, and participating in various life processes, such as a metabolic turnover, a cell cycle, morphogenesis, a neurological function, and an immune response, deeply is known. This system is saved over the living thing kind at altitude, and consists of the following processes until it results [from yeast] in Homo sapiens. That is, Gly residue of a C terminal is adenyl-ized by ubiquitin activating enzyme (UBE1 or UBA) and ATP, and carries out thioester association of the ubiquitin by them at the specific Cys residue of UBE1. Subsequently, the activated ubiquitin is transferred to the specific Cys

residue of a ubiquitin joining enzyme (UBE2 or UBC), and carries out thioester association. Ubiquitin-ization of a protein matrix may be performed through the case where it is directly carried out from UBE2, and a ubiquitin ligase (UBE3 or UBR), and ubiquitin carries out the isopeptide bond also of any to the Lys residue of a protein matrix. Furthermore, ubiquitin combines with the Lys residue of the ubiquitin intramolecular combined with the protein matrix one after another, the poly ubiquitin chain is formed, and a protein matrix is eventually decomposed by proteasome. UBE2 which is a ubiquitin joining enzyme, and UBE3 which is a ubiquitin ligase are understood that the selectivity or the singularity of a ubiquitin system is specified by forming a gene family and making specific protein into a substrate, respectively.

[0005] In the process which carries out large quantity analysis of the Homo sapiens cDNA clone previously, this invention persons isolated the gene of the new enzyme considered to be one of the ubiquitin joining enzymes UBE2, and named this enzyme UBE2G (JP,9-308492,A). In northern blot analysis, mRNA of Homo sapiens UBE2G was strongly discovered by skeletal muscle, and also the feeble manifestation was detected in all the organizations that investigated. The presumed amino acid sequence consisted of 170 amino acid residue, and the Cys residue made indispensable respectively in accordance with UBC7 of yeast and a nematode at 52% and 76% of a rate at ubiquitin joining enzyme (UBE2) activity covering an overall length was also saved. Moreover, it was shown by the experiment using recombination protein that UBE2G carry out thioester association with ubiquitin at a UBE1 dependence target. Therefore, from these things, it was checked that UBE2G are a new ubiquitin joining enzyme. In addition, in the description concerned, the gene which carries out the code of this enzyme for these ubiquitin joining enzyme UBE2G to UBE2G1 is called UBE2G1 gene.

[0006] UBE2G1 was discovered very weakly in all the organizations that examined, although it was strongly discovered especially in skeletal muscle. Moreover, all other ubiquitin joining enzymes (UBE2) are related to an indispensable cell function like accommodation of a cell cycle, and have discovered them across an organization. However, UBE2G1 was participating in disassembly of muscle specific protein, and it was thought that the incompetence of this gene became a disease like myotrophia dystonica as a result.

[0007] However, it is not fully solved about these operations, but many points which should be solved are left behind about work of UBE2G1 in the living body. Although it craves as the so-called model of a UBE2G1 gene-expression insufficient animal which does not produce UBE2G1 at all, or produces only small quantity when solving the operation in the living body of UBE2G1 being useful, such animal model is not created in this time.

[0008]

[Problem(s) to be Solved by the Invention] This invention aims at offering a useful model animal, i.e., a UBE2G1 genetic-defect nonhuman animal, in order to study the physiological role of UBE2G1, and the approach of clarifying especially the operation to the skeletal muscle, and solving the various symptoms in which UBE2G1 participates in skeletal muscle, or treating these symptoms. Moreover, this invention aims at offering the above-mentioned UBE2G1 genetic-defect nonhuman animal as a mesangium hardening model.

[0009]

[Means for Solving the Problem] In order to solve the above-mentioned technical problem, when this invention persons were inquiring wholeheartedly constantly, they found out that the nonhuman animal which UBE2G1 gene was destroyed, consequently lost ubiquitin joint enzyme activity was obtained by building the specific vector which destroys specifically a Homo sapiens ubiquitin joining enzyme (UBE2G1) gene using genetic manipulation, and making this and homonous recombination cause. this invention persons specifically use the homologous gene in the mouse of UBE2G1 gene. Build the specific vector which destroys UBE2G1 gene specifically, and this is introduced into the undifferentiated cell which has totipotency embryologically. Subsequently, select the cell by which UBE2G1 gene was destroyed, and it pours in to an animal germ. By transplanting to the uterus of assumed parents, acquiring the chimera animal of a germ cell line, making this chimera animal cross with a normal animal, creating a hetero deficit mold animal (+/-) and making hetero deficit molds cross further The both sides of a gay deficit mold animal (-/-), i.e., a locus, checked that a transgenic nonhuman animal to have alteration UBE2G1 gene which does not carry out the code of UBE2G1 substantially was obtained. For this invention, all or a part of UBE2G1 genes which carries out the code of the : (1) ubiquitin joining enzyme UBE2G1 which is what includes invention which is completed based on this knowledge and hung up over following the (1) - (7) are the UBE2G1 genetic-defect nonhuman animals which come to lose ubiquitin joint enzyme activity substantially by having inserted or permuted other genes by deletion or the arbitration part.

(2) A UBE2G1 genetic-defect nonhuman animal given in (1) whose gene which carried out deletion is the 2nd exon of UBE2G1 gene.

(3) A UBE2G1 genetic-defect nonhuman animal given in (1) other genes of whose are neomycin resistance genes.

(4) (1) which is a gay deficit mold or a hetero deficit mold thru/or (3) — a UBE2G1 genetic-defect nonhuman animal given in either.

(5) A UBE2G1 genetic-defect nonhuman animal given in either [which is a gay deficit mold] (1) thru/or (3).

(6) A UBE2G1 genetic-defect nonhuman animal given in (5) which is a

mesangium hardening model animal.

(7) A UBE2G1 genetic-defect nonhuman animal given in either [whose a nonhuman animal is a mouse] (1) thru/or (6).

[0010]

[Embodiment of the Invention] The display by codes, such as amino acid in a following and book description, a peptide, a base sequence, and a nucleic acid, is convention [IUPAC-IUB Communication on Biological Nomenclature of IUPAC-IUB, Eur.J.Biochem., and 138. : The common use notation in 9 (1984)], "the guideline for creation, such as a description including a base sequence or an amino acid sequence," (edited by Patent Office), and the field concerned shall be followed.

[0011] moreover, [Molecular Cloning 2d Ed which can perform easily the manufacture approach of the manifestation protein which the host cell and host cell by which the transformation was carried out secrete etc. according to them according to the general gene engineering-technique by composition of DNA, manufacture of the vector (expression vector) containing an exogenous gene, and this vector and Cold Spring Harbor Lab.Press; (1989) New Biochemistry Experiment Lectures -- reference, such as "the gene approaches I, II, and III" and the edited by etc. Japanese Biochemical Society (1986), --] .

[0012] In this invention, a gene is used with the meaning which all includes 1 each chain DNA called not only the double stranded DNA but the sense chain and antisense strand which constitute it, and is not restricted to the die length at all. both [therefore,] the double stranded DNA which contains the genomic DNA of mammalian unless especially the gene of this invention is mentioned and the single stranded DNA (sense chain) containing cDNA the single stranded DNAs (antisense strand) which have this sense chain and a complementary array in a list and those fragments -- although -- it is contained.

[0013] Moreover, in this invention, a gene does not ask exceptions, such as a leader sequence, a coding region, an exon, and the intron, and includes all. Moreover, RNA and DNA are illustrated as a polynucleotide. Each of the equivalent effectiveness objects (a homolog, a derivative, variant) is contained in the peptide which each of cDNA(s), genomic DNA, and synthetic DNAs is contained in DNA, and consists of a specific amino acid sequence (Pori).

[0014] In addition, the variant which creates artificially the gene of the variant (for example, allele variant) produced by mutation, qualification after a translation, etc. in nature and the natural origin or (Pori) the arbitration part of the array of a peptide deletion, a permutation, or by making it add (or insertion) is included by the variant (a gene, peptide (Pori)).

[0015] UBE2G1 gene as used in the field of this invention is a gene of the

non-Homo sapiens who does the code of ubiquitin joining enzyme UBE2G1, and can mention the gene of the mouse which carries out the code of the ubiquitin joining enzyme UBE2G1 which consists of an amino acid sequence shown in the array number 1 as an example. However, ubiquitin joining enzyme UBE2G1 also includes the homologous object which has the same activity as the ubiquitin joining enzyme concerned, without being limited to the amino acid sequence shown in the array number 1. "The homologous object of UBE2G1" has UBE2G1 and sequence homology, and means a series of one related protein recognized to be familiar with the similarity in a gene expression pattern, and the similarity of biological functions in the structural description list here, and a series of protein generated from an allele object (allele) is also contained. Moreover, the protein originating in rodent animals, such as mammalian, for example, Homo sapiens, a horse, a sheep, a cow, a dog, an ape, a cat, a bear, a rat, and a rabbit, which has the same activity as UBE2G1 which has the amino acid sequence shown in the array number 1 is also included by the homologous object of UBE2G1.

[0016] The gene which has the base sequence shown in the array number 2 as a concrete mode of UBE2G1 gene can be illustrated. This base sequence is one example of combination of the codon which shows each amino acid residue of the amino acid sequence shown in the array number 1. If an example is taken by the degeneracy of a codon, not only the gene that has this specific base sequence but UBE2G1 gene can have combination and the selected base sequence for the codon of arbitration to each amino acid residue. Selection of a codon can take into consideration the codon usage of the host who can follow a conventional method, for example, uses etc.

[Nucleic Acids Res., 9, and 43 (1981)].

[0017] [Molecular Cloning 2d Ed which UBE2G1 gene can be based on the array information on UBE2G1 gene indicated by this invention, and can be easily manufactured and acquired by the general gene engineering-technique, and Cold Spring Harbor Lab.Press; (1989) New Biochemistry Experiment Lectures -- reference, such as "the gene approaches I, II, and III" and the edited by etc. Japanese Biochemical Society (1986), --] .

[0018] From the suitable origin where UBE2G1 gene is discovered, a cDNA library is prepared according to a conventional method, and, specifically, it can carry out by choosing a request clone from this library using a suitable probe and a suitable antibody peculiar to UBE2G1 gene [Proc.Natl.Acad.Sci., USA., 78, 6613;(1981) Science, 222, 778 (1983), etc.].

[0019] In the above, various kinds of cells which discover UBE2G1 gene, the cultured cell originating in an organization or these, etc. are illustrated as the origin of cDNA. Moreover, each can carry out separation of all RNA from these, separation of mRNA, purification, acquisition, cloning, etc. of cDNA, etc. according to a conventional method. Moreover, a cDNA library can also

use the various cDNA libraries which are also marketed and are marketed from these cDNA(s) library (Clontech Lab.Inc.), for example, Clonetec, in this invention.

[0020] The approach of screening UBE2G1 gene from a cDNA library is not restricted especially, either, but can follow the usual approach.

[0021] Plaque hybridization, colony hybridization, etc. using the approach of choosing the cDNA clone which corresponds by immunity-screening which specifically used the specific antibody of this protein to the protein produced by cDNA, and the probe selectively combined with the target DNA array, these combination, etc. can be illustrated.

[0022] Although DNA by which chemosynthesis was carried out based on the information about the base sequence of UBE2G1 gene as a probe used here can generally use it, UBE2G1 already acquired gene and its fragment can also be used good. Moreover, the sense primer and antisense primer which were set up based on the base sequence information on UBE2G1 gene can also be used as a probe for screening.

[0023] the partial nucleotide sequence corresponding to the array number 2 in the nucleotide sequence used as said probe -- it is -- at least 15 continuous bases -- 20 continuous bases and electropositive clone itself which 30 continuous bases and the thing which has 50 continuous bases most preferably are also contained more preferably, or has said array can also be preferably used as a probe.

[0024] On the occasion of acquisition of UBE2G1 gene, the DNA/RNA amplifying method by the PCR method [Science, 230, and 1350 (1985)] can use suitably. a case so that cDNA of an overall length may especially be hard to be obtained from a library -- the RACE method [the Rapid amplification of cDNA ends; experimental medicine, 12 (6), and 35 (1994)] -- adoption of the 5'-RACE method [M.A.Frohman, et al., Proc.Natl.Acad.Sci., USA., 8, and 8998 (1988)] etc. is especially suitable.

[0025] The primer used on the occasion of adoption of this PCR method can be suitably set up based on the array information on UBE2G1 gene clarified by this invention, and this can be compounded according to a conventional method. In addition, isolation purification of the DNA/RNA fragment made to amplify can follow a conventional method as aforementioned, for example, should just be based on gel electrophoresis etc.

[0026] Moreover, UBE2G1 gene or the various DNA fragments which are obtained above can determine the base sequence using a commercial sequence kit etc. according to a conventional method [Proc.Natl.Acad.Sci., USA., 74, and 5463 (1977)], for example, a dideoxy chain termination method, the Maxam-gilbert method [Methods in Enzymology, 65, and 499 (1980)], etc. simple.

[0027] This invention with the UBE2G1 genetic-defect nonhuman animal

which lost the target ubiquitin joining enzyme (UBE2G1) activity By adding variation (deletion, a permutation, addition, insertion) to the UBE2G1 genome gene of mammals other than Homo sapiens artificially The activity of UBE2G1 in which this gene expression ability controls or loses, or this gene carries out a code is the nonhuman animal (henceforth a knockout animal) which does not discover ubiquitin joining enzyme (UBE2G1) activity substantially by controlling or losing substantially.

[0028] Here, although GETSU gear-tooth animals, such as ontogeny and a mouse with comparatively easy propagation with a lifelong short and cycle, and a rat, are desirable as a nonhuman animal when creating the animal model, it is not limited to especially this and the animal of the mammals, such as rabbits other than Homo sapiens, Buta, a sheep, a goat, and a cow, is also contained in this.

[0029] It can carry out by carrying out deletion of all of the base sequences of this gene by the gene engineering-technique, or carrying out deletion of a part of base sequence of the above-mentioned genome gene, or inserting other genes in the part of arbitration, or permuting [**** / shifting the reading frame of a codon] with other genes as an approach of adding variation to the UBE2G1 genome gene as used in the field of above artificially, so that the function of a promotor or an exon may be destroyed. The above-mentioned artificial variation approach For example, site SUPESHIFIKKU mu factor TAGENESHISU [Methods in Enzymology, 154, 350, and 367-382; (1987) ** 100 and 468; (1983) Nucleic Acids Res., 12, and 9441; (1984) New Biochemistry Experiment Lectures 1 "the gene approach II", The gene engineering-technique, such as the edited by Japanese Biochemical Society and p105 (1986)], chemosynthesis means [J.Am.Chem.Soc., such as a phosphotriester method and a phosphoric-acid aminodite method, 89, and 4801; (1967) — said — 91, 3350; (1969) Science, and 150 — 178; (1968) Tetrahedron Lett., 22, 1859 (1981); ** It can carry out 24, 245 (1983)], and by [those] combining.

[0030] More specifically the knockout animal of this invention For example, the UBE2G1 genome gene of the target nonhuman mammal is isolated. Into the exon part, a neomycin resistance gene (henceforth neo), the drug resistance gene which makes a hygromycin tolerance gene representation — or — It lacZ(s) (beta-galactosidase gene). [whether the function of an exon is destroyed by inserting the reporter gene which makes CAT (chloramphenicol acetyltransferase gene) representation, and] Or the DNA array which makes the intron part between exons end the imprint of a gene The DNA strand which has the DNA array built so that a gene might be destroyed as a result by (inserting [for example,] a polyA addition signal etc.) and carrying out by the ability not compounding perfect messenger RNA It introduces into the chromosome of this animal cell for example, by the

electroporation method (hereafter abbreviated to a targeting vector). Into the obtained recombination embryonic stem cell It attaches. The DNA array of a UBE2G1 gene top or its near In the analysis by the PCR method which made the primer the DNA array on the Southern hybridization analysis used as the probe, or a targeting vector, and the DNA array of near fields other than the UBE2G1 gene used for targeting vector production It can obtain by sorting out a UBE2G1 knockout embryonic stem cell.

[0031] When inserting other genes in the above, it is desirable to insert other genes which function also as a selective marker for detecting the variation of UBE2G1 gene, and a neomycin resistance gene (neo) can illustrate preferably as such a gene. In this case, the judgment of recombination having arisen is attained by acquisition of neomycin (or G418 which is that analog) resistance. In order to remove a random recombination object furthermore, it is desirable to introduce the gene which has lethality to a cell like a diphtheria toxin A fragment gene (henceforth "DT-A") on the outside of a homologous part so that positive-negative sorting may be attained. In this way, a lethal gene is introduced by random recombination and the targeting vector obtained becomes fatal [such a cell]. Therefore, it becomes possible by performing positive-negative sorting to acquire a homologous recombination object efficiently. Insertion of these genes can be performed by DNA recombination technique in ordinary use within a test tube.

[0032] In operation of this invention, the 2nd exon of UBE2G1 gene is carrying out deletion, and it is desirable that the selective marker gene is inserted in the deletion field. In this invention specifically As a positive selective marker MC1-neo-polyA (a neomycin resistance gene and an SV40 origin poly A addition signal on MC1 promotor's (promotor who consists of a Polyoma virus origin variation enhancer sequence and a thymidine kinase gene promotor array) lower stream of a river) What was connected : Thomas and KR.& Capecchi.M.R.: Site-directed mutagenesis by gene targeting in mouse embryo-derived stem cells.: Cell, 51, and 503 (1987), and as a negative selective marker MC1-DT-A () [thing:Yagi by which the diphtheria toxin A fragment was connected with MC1 promotor's lower stream of a river,] [T.et al.:A novel negative selection for homologous recombinants] using diphtheria toxin A fragment: Analyt Biochem., 214, and 70 (1993) are used suitably.

[0033] In the above-mentioned actuation a mouse UBE2G1 genome gene For example, a mouse genomic DNA library is screened by using a well-known Homo sapiens UBE2G1 cDNA array (equivalent to the example 7 of JP,9-308492,A, and the array number 23 (ORF field of Homo sapiens UBE2G)) as a probe. The electropositive clone containing a protein coding region is isolated, and it digests with a restriction enzyme after inserting this in a vector, and can acquire by carrying out subcloning of the chromosome

DNA containing a specific exon.

[0034] Creation of a mouse genomic DNA library is the lab manual gene engineering (the volume Maruzen Co., Ltd. issuance and for Masami Muramatsu, 1990) 100-112. Being able to carry [and] out according to an approach given in a page, creation of mouse UBE2G1cDNA is the lab manual gene engineering (the volume Maruzen Co., Ltd. issuance and for Masami Muramatsu, and 1990) 83-99. It can carry out according to an approach given in a page.

[0035] moreover, as 1 ascertainment of whether mouse UBE2G1 is the homologous object of Homo sapiens UBE2G1 By RADIESHON hybrid mapping (Radiation Hybrid Mapping) Chromosome mapping of a mouse UBE2G1cDNA clone is performed (Cox, D.R., et al., Science, 250, and 245-250 (1990)). The chromosome seating position of mouse UBE2G1 gene, It is good to judge by whether the chromosome seating position where a Homo sapiens homologous gene exists is the region of homology.

[0036] Moreover, for the culture embryonic stem cell of 129 systems, the transgenics to a mouse embryonic stem cell is a 129/Sv mouse origin genomic library in acquisition of a genomic DNA clone, when it mostly comes out and a certain thing is taken into consideration. (Stratagene make: Stratagene) It is more desirable to use as a probe the mouse UBE2G1cDNA clone obtained above.

[0037] In this way, the genomic DNA clone which contains the 2nd exon from the above-mentioned library is obtained, and the structure of the gene field made into the object using this for production of a targeting vector is destroyed. the after-mentioned example of this invention -- setting -- as the region of homology -- the PstI-EcoRV fragment (0.8kb) of the 2nd exon upstream, and a down-stream XbaI-EcoRI fragment (9.5kb) -- moreover, MC1-DT-A (life tech oriental company make) can be used as MC1-neo-polyA (Stratagene make) and a negative selective marker as a positive selective marker, respectively, and a homologous permutation can be carried out.

[0038] In addition, the mouse UBE2G1 genome gene used as the target for ubiquitin joining enzyme genetic defect nonhuman animal creation of this invention is a gene which consists of at least six exons. Although the neomycin resistance gene is made to carry out the homologous permutation of the field which contains the 2nd exon part in the example of this invention, genes, such as a neomycin resistance gene, may be made to carry out a homologous permutation combining other either of the exon parts or two or more place exon parts, or deletion may be carried out.

[0039] Subsequently, what could use the already established above things, for example as an embryonic stem cell of the origin to which inactivation of the UBE2G1 gene is carried out by the homologous rearranging method etc.,

and was newly established according to the approach of well-known Evans and Kauffmann may be used.

[0040] For example, in the case of the embryonic stem cell of a mouse, generally the embryonic stem cell of 129 systems is used, but For the object of a genetic background acquiring a clear embryonic stem cell immunologically by the pure line strain which changes to this, since the immunological background has not clarified, for example the little of C57BL/6 mouse or the number of egg gathering of C57BL/6 -- the intersection of DBA/2 -- what was established using BDF1 mouse (F1 of C57BL/6, and DBA/2) improved more coarsely can be used good. Since the number of egg gathering has many [and] BDF1 mice against the background of C57BL/6 mouse in addition to the advantage that an egg is strong, the embryonic stem cell obtained using this can be advantageously used at the point which can change the genetic background into C57BL/6 mouse by carrying out a back cross with C57BL/6 mouse, when a symptoms model mouse is created.

[0041] Moreover, although a carrier Seigo 3-5 day blastocyst is generally used when establishing an embryonic stem cell, it is also possible by gathering the eggs of 8 cell term germ, and cultivating and using to a blastocyst in addition to it, to acquire many early embryos efficiently. moreover, a sex -- although which embryonic stem cell may be used, it is convenient for the direction of a male embryonic stem cell usually creating a germ cell line chimera.

[0042] In order to introduce a targeting vector into an embryonic stem cell, it is necessary to carry out culture maintenance of the embryonic stem cell in the undifferentiated condition so that the differentiation potency to a reproductive cell may be maintained. For that purpose, it requires adding the suitable vegetative cell for a culture medium as a feeder cell, and adding Homo sapiens LIF (LeukemiaInhibitory Factor), adding fetal calf serum, a nucleoside, nonessential amino acid, 2-mercaptoethanol, etc. further, and cultivating. As a vegetative cell, a STO cell or mouse fetus fibroblast can be illustrated preferably.

[0043] The addition concentration of a feeder cell has desirable 2.5×10^5 cells / ml (activity of 10ml / 100mm culture plate, 2ml / 35mm culture plate, 4ml / 60mm culture plate, 0.5 ml/well:24-well plate, etc.). Although Homo sapiens's LIF addition concentration has desirable 103 units / ml, more than it is sufficient as it. The addition concentration of a nucleoside has [the addition concentration of fetal calf serum] desirable 10-30nM 20%. As nonessential amino acid, the Gibco nonessential-amino-acid solution is desirable, and the concentration has 0.1 desirable mM(s) each, for example. The concentration of 2-mercaptoethanol has desirable 0.1mM(s). In order to introduce a targeting vector into an embryonic stem cell by the electroporation method, it is desirable to make the buffer solution float so that it may become fixed

concentration about an embryonic stem cell, to add the targeting vector which processed with the suitable restriction enzyme and was linearized, and to perform electroporation by 270V / 1.8mm or 250v / 4mm, and 500microFD, using suitable electroporation equipments, such as ECM600 (BTX company make) or a gene pulsar (biotechnology rad company make). It is desirable to adjust pH to 7.0, using PBS etc. as the buffer solution. A restriction enzyme is chosen in consideration of the restriction enzyme part of a targeting vector.

[0044] After introducing a targeting vector, an embryonic stem cell is cultivated by the above-mentioned culture medium for 24 to 48 hours, and it exchanges for the culture medium containing G418 (JENNETISHIN: product made from Geneticin:Gibco BRL) after that. It exchanges for new G418 addition culture medium day by day [1], and culture is continued for about one week.

[0045] The clone of G418 resistance which introduced the above-mentioned targeting vector and was acquired is picked up, and it checks by isolating a homologous recombination object.

[0046] First, the above-mentioned drug tolerance embryonic stem cell is proliferated, and DNA is extracted from the part. Detection of a homologous recombination object is checked by the Southern hybridization method as mentioned above.

[0047] After performing restriction enzyme processing after extracting genomic DNA and performing agarose electrophoresis, Southern hybridization is performed for the UBE2G1 gene DNA to a probe. In this way, if the fragment of a targeting vector is detectable, it will mean that it was proved that the embryonic stem cell is the clone from which one allele of target UBE2G1 gene started homologous recombination with the targeting vector.

[0048] Subsequently, creation of a transgenic nonhuman animal which has the alteration gene by which the chimera animal of a genital system and UBE2G1 gene were destroyed is performed as follows.

[0049] First, a hetero deficit mold embryonic stem cell is poured into the germ of an animal, and it transplants to it at the uterus of assumed parents. Here, as for the germ of an animal, a blastocyst is used. It is because it is suitable for formation of a chimera. About 10-15 embryonic stem cells are usually poured in. In this way, the germ into which the embryonic stem cell was poured is promptly transplanted to the uterus of assumed parents.

[0050] A chimera animal is chosen among the children borne by the assumed parents conceived by the above-mentioned approach. Although the high chimera animal of the contribution of an embryonic stem cell has high possibility of being the chimera animal of a germ cell line, the check of being the chimera animal of a germ cell line is possible by crossing a chimera animal with the normal animal. A hetero deficit mold animal is obtained by

mating with the chimera animal of a germ cell line, and the normal animal, and the gay deficit mold animal for which both UBE2G1 alleles were destroyed by mating of hetero deficit molds can be obtained.

[0051] Thus, the transgenic nonhuman animal which is an obtained gay deficit mold animal is missing, and can use UBE2G1 with a role important for formation of a mesangium lesion as the animal used in disease modeling, especially renal failure model of mesangium lesion formation.

[0052] The UBE2G1 gene-expression insufficient nonhuman animal of this invention As opposed to the animal of the same kind which has UBE2G1 normal gene by inactivation of UBE2G1 gene Are the nonhuman animal by which the amount of gene expression was made to decline, for example, a field to the ontogeny and biocycle of production of a model-animal-of-pathosis system are comparatively short. Moreover, although GETSU gear-tooth animals, such as a mouse with easy propagation and a rat, are desirable, not only these but a rabbit, Buta, a cow, a goat, a sheep, a dog, a cat, etc. are especially contained in this.

[0053] the UBE2G1 gene-expression incompetence (UBE2G1 knockout) nonhuman mammal of this invention -- for example, the targeting vector produced as mentioned above can be introduced into a mouse embryonic stem cell or a mouse ootid, and UBE2G1 gene can be made to destroy, when the UBE2G1 gene fragment with which the targeting vector was inactivated replaces UBE2G1 gene on the chromosome of a mouse embryonic stem cell or a mouse ootid by gene homologous recombination

[0054] The cell by which UBE2G1 gene was destroyed can be judged in the analysis by the PCR method which made the primer the DNA array on the Southern hybridization analysis which used the DNA array of a UBE2G1 gene top or its near as the probe as mentioned above, or a targeting vector, and the DNA array of near fields other than the mouse UBE2G1 gene used for the targeting vector.

[0055] Moreover, the approach of obtaining the UBE2G1 manifestation nonhuman animal permuted by the reporter gene in the structural gene of UBE2G1 as an option which makes UBE2G1 gene inactivating can be illustrated. In this approach, since a reporter gene exists under rule of the promotor of UBE2G1, UBE2G1 promotor's activity is detectable by tracing the manifestation of the matter in which a reporter gene carries out a code. For example, when a part of gene field which carries out the code of the UBE2G1 protein is permuted with the beta-galactosidase gene (lacZ) of the Escherichia coli origin, the beta-galactosidase is discovered instead of UBE2G1 in the organization which original UBE2G1 discovers. The manifestation condition of UBE2G1 animal in the living body is observable simple by dyeing using the reagent which follows, for example, serves as a substrate of beta-galactosidase like

5-BUROMO-4-chloro-3-indolyl-beta-galactopyranoside (X-gal). It is the stain solution which fixes a UBE2G1 deficit mouse or its organization intercept by glutaraldehyde etc., and specifically contains X-gal after washing with a Dulbecco phosphoric-acid buffer physiological salt solution (PBS), is a room temperature or near 37 degree C, and is 1mM about the preparation about 30 minutes thru/or after making it react for 1 hour. What is necessary is to stop a beta-galactosidase reaction and just to observe coloration by washing with an EDTA/PBS solution. Moreover, a conventional method is followed and it is lacZ. mRNA may be detected. Such a UBE2G1 manifestation insufficient nonhuman animal of this invention is very useful when screening activation or the matter which carries out inactivation, and it can contribute UBE2G1 promotor to cause investigation of various diseases, such as renal failure resulting from UBE2G1 manifestation incompetence, or development of a remedy greatly.

[0056] The renal failure model by mesangium hardening formation of this invention does not have the deposition of an immunoglobulin, and extracellular matrices, such as the collagen type IV and a tee necine, are the nonhuman model animals considered that a mesangial cell is activated by a certain mechanism since an increment and the increment in the manifestation of alpha-smooth muscle actin in the glomerulus to which the lesion reached an advanced stage are seen, and production of an extracellular matrix is accelerating.

[0057] Although the glomerulus consists of an epithelial cell, an endothelial cell, a mesangial cell, glomerular basement membrane, and a mesangium substrate, the increment in a mesangium substrate is called glomerulosclerosis and the minimum arteriosclerosis, glomerulonephritis, a diabetic nephritis, etc. are regarded as an end image of all glomerulus failures, about the onset mechanism, it is not fully solved. Therefore, the UBE2G1 genetic-defect mouse of this invention is useful as a new model of mesangium hardening.

[0058]

[Effect of the Invention] The UBE2G1 genetic-defect nonhuman animal obtained by this invention, especially the gay deficit mold mouse are missing, and can use effectively UBE2G1 with a role important for formation of a mesangium lesion as the animal used in disease modeling, especially renal failure model of mesangium lesion formation.

[0059]

[Example] Although an example is given to below and this invention is concretely explained to it, it cannot be overemphasized that this invention is not limited to these.

Out of the mouse EST released by the production Genbank of the vector for example 1(1) UBE2G1 gene disruption, what consists of an array similar to

the array of a Homo sapiens UBE2G gene protein coding region is searched by the BLAST program, they are connected using the C quencher (Gene Codes Corporation), and it is mouse UBE2G1. The virtual array of cDNA was determined. The PCR reaction which compounded oligonucleotide 423A12P7 (array number 4) and 423A12P8 (array number 5), and used the mouse skeletal muscle origin cDNA (Marathon-Ready cDNA, Stratagene make) as mold by making these into a primer based on this array information was performed, and the cDNA clone containing a protein coding region was isolated. This obtained clone was named the mouse UBE2G1 cDNA clone.

[0060] The gene of mouse UBE2G1 has the ORF field which carries out the code of the new ubiquitin joining enzyme which consists of 170 amino acid sequences shown in the array number 1 (510 base sequences, array number 2). In addition, a cDNA array (1274 bases) including 5', and 3' untranslation region and a protein translation field is shown in the array number 3. The homology of this mouse UBE2G1 cDNA clone and a Homo sapiens UBE2G cDNA clone is 95% on nucleic-acid level, and the amino acid sequence presumed was thoroughly in agreement.

[0061] Furthermore, RADIESHON hybrid mapping (Radiation Hybrid Mapping) performed chromosome mapping of a mouse UBE2G1 cDNA clone (Cox, D.R., et al., Science, 250, and 245-250 (1990)).

[0062] That is, the mouse T31 RADIESHON hybrid panel was purchased from the research FTgenetics company (Research Genetics, Inc., AL, U.S.A.), and the PCR reaction was carried out using M423A12 RH-P3 primer (array number 6) and M423A12 RH-P6 primer (array number 7) which consist of a base sequence shown below according to the directions for use of a product.

[0063] m423A12RH-P3 primer 5 -- '-TGTTTACATATAACCTCACC-3' -- usable software analyzed the obtained result on the Internet :

5'-GACAATGATCTCTATCGTTG-3' m423A12 RH-P6 primer: (WI/MIT Mouse Radiation Hybrid Mapper

(<http://www.genome.wi.mit.edu/cgi-bin/mouse#rh/rhmap-auto/rhmapper.cgi>.)

Consequently, mouse UBE2G1 gene was located in about 320 mouse chromosome marker D11Mit, and this field was the region of homology in the mouse chromosome of the chromosome 17p13 in which a Homo sapiens homologous gene exists.

[0064] From the above result, it was judged that this gene was UBE2G1 gene of a mouse.

[0065] (2) Use the production Homo sapiens UBE2G1 gene cDNA of a targeting vector as a probe, and they are 129SV / mouse origin genomic library. (Stratagene make: Stratagene) As a result of screening, the genomic DNA clone containing the 2nd exon was obtained. production of a targeting vector -- as the region of homology -- the PstI-EcoRV fragment (0.8kb) of

the 2nd exon upstream, and a down-stream XbaI-EcoRI fragment (9.5kb) -- moreover, the diphtheria toxin A fragment gene (Stratagene make) connected with MC1 promotor as a positive selective marker as MC1NeoPolyA (life tech oriental company make) and a negative selective marker was used, respectively, and this was built as a targeting vector (refer to drawing 2 A).

[0066] Example 2 As a cell which introduces the targeting vector of mouse UBE2G1 gene produced according to the culture example 1 of the production (1) mouse embryonic stem cell of a UBE2G1 genetic-defect mouse The STO cell (it receives from the University of Tokyo and Professor Motonari Katsuki) which used and carried out the transformation of the embryonic stem cell CCE stock (it receives from Professor Harvard University E.J.Robertson) established from the 129 system mouse blastocyst by neo as a feeder cell of an embryonic stem cell was used. Culture of an embryonic stem cell is "Teratocarcinomas and embryonic stemcells. : It carried out according to a practical approach, p.71-151, and IRL Press.Oxford." (E. J.Robertson, 1987).

[0067] 1-N hydrochloric acid after adding 44mM sodium hydrogencarbonate (life tech oriental company make) was added to the Dulbecco alteration Eagle's medium (life tech oriental company make), it adjusted to pH7.2, and this was made into the basal medium. The culture medium which added foetal calf serum (life tech oriental company make), penicillin, and streptomycin (respectively 50 units / ml, and 50microg [ml] / , life tech oriental company make) 10% to the basal medium concerned was used for culture of a STO cell. In culture of an embryonic stem cell, they are 20% foetal calf serum (life tech oriental company make) and 0.1mM to a basal medium.

beta-mercaptoethanol, 0.1mM Nonessential amino acid (life tech oriental company make), 30nM adenosine, 30nM guanosine, 30nM cytidine, 30nM uridine, 10nM thymidine (above wholly sigma company make), and the culture medium that added ESGRO (1000 units/ml, the product made from AMURADO; AMRAD) were used.

[0068] Seeding of the STO cell is carried out by the concentration of 1.2×10^5 cells per 100mm culture plate. Carrying out the passage of the part three days after, the remainder is 2-hour mitomycin-C (10 ng/ml) four days after. 2.5×10^5 after processing by sigma company make cells / concentration of ml -- a culture plate -- pouring (a 10ml/100mm culture plate --) 4ml / 60mm It was used as a feeder cell from a culture plate, 2 ml/well:6-well plate, 0.5 ml/well:24-well plate, and its next day. Seeding of the embryonic stem cell was carried out on the feeder cell by 1.5×10^5 cells / concentration of ml (10ml/100mm a culture plate, and 4ml / 60mm culture plate), it carried out culture-medium exchange at the next day, and carried out the passage two days after.

[0069] (2) Targeting vector 50microg which 1ml PBS (pH7.0) was made to

float, and line-ized the production CCE cell of a chimeric mouse and a UBE2G1 gene-disruption mouse by restriction enzyme SalI digestion was introduced into 3.0×10^7 embryonic stem cells by electroporation. The equipment and materials used for transgenics were set to ECM600 (BTX Inc. company make), and conditions were set to 270V / 1.8mm, and 500 micro F. It is JIENETISHIN (Geneticin) (250microM, the product made from Gibco BRL: GibcoBRL) in 48 hours after installation. It added, drugs selection was performed for seven days after that, and 120 clones were collected out of 2161 JIENETISHIN resistance colonies. As a result of extracting genomic DNA from these embryonic stem cells and performing southern blot analysis using below-mentioned 5' probe and 3' probe and a below-mentioned neo probe, seven shares were identified as homologous recombinant. Three shares (clone numbers 9, 38, and 115) were poured in to C57BL / 6J mouse blastocyst among these, and the chimeric mouse was produced.

[0070] That is, the CCE cell in which one UBE2G1 allele was destroyed by the above targeting was injected into C57BL / 6 system mouse (it receives from Japanese Clare, Inc.) blastocyst, and was transplanted to the uterus of assumed parents. 10-15 CCE cells were poured in here. Consequently, 266 germs were transplanted to the assumed parents of 13 animals, and the child of 34 animals was obtained. From the color of hair, seven animals were chimeric mice among those (four males, three females). When the back cross of the male of a chimeric mouse and the female of C57BL/6J mouse (it receives from Japanese Clare, Inc.) was carried out, the chimera of a germ cell line was checked with the male chimeric mouse, and the hetero deficit mold mouse (+/-) with which one UBE2G1 allele was destroyed by targeting was obtained. The male and female of a hetero deficit mold mouse (+/-) were crossed, and the gay deficit mold mouse (-/-) with which both alleles were destroyed was produced.

[0071] The genotypes of F2 mouse obtained by mating between F1 hetero mice as shown in a table 1 were 64 wild type mice (+/+) (37 males, 27 females), 108 hetero deficit mold mice (+/-) (52 males, 56 females), and 50 gay deficit mold mice (-/-) (18 males, 32 females).

[0072]

[A table 1]

| | +/+ | +/- | -/- |
|----|-----|-----|-----|
| ♀ | 27 | 56 | 32 |
| ♂ | 37 | 52 | 18 |
| 合計 | 64 | 108 | 50 |

[0073] The UBE2G1 gene hetero deficit mold mouse (+/-) was born normally, and was also able to be bred. On the other hand, even if the weight of the UBE2G1 gene gay deficit mold mouse (-/-) of 6 weeks old of after the birth was small about 20% compared with the wild type (+/+) and it became age for six months, it did not catch up (drawing 3). However, except for this point, the exterior difference was not found with a gay deficit mold mouse (-/-) and a wild type mouse (+/+) in [after the birth] one year.

[0074] The fertilized egg of the obtained UBE2G1 gene hetero deficit mold mouse is deposited with National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology, the Ministry of International Trade and Industry, (NIBH) which does the location to 1-1-3, Higashi, Tsukuba-shi, Ibaraki-ken as the display for the discernment as of August 24, Heisei 12 which it is and the bailor attached "a UBE2G (423A12) genetic defect mouse origin fertilized egg", and a trust number "FERMP-17997" (advice number: 12 student ***** No. 1114).

[0075] Example 3 5'3 located in probe (Spe I-Sca I fragment, 0.6kb) and lower stream of a river of the region of homology' probe (an EcoR I fragment, 1kb) located in the upstream of the region of homology used for construction of a targeting vector was used for the judgment of the judgment genotype of genotype. . Mouse genomic DNA was digested with the restriction enzyme KpnI, and Southern blotting hybridization was performed according to the conventional method (Maniatis et al.1990). At this time, variant allele was detected by 5' probe as a fragment of 14.5kbs with 7.5kbs and 3' probe to wild type allele being detected as a fragment of 21kbs also in any of 5' probe or 3' probe (drawing 2 , B).

[0076] Example 4 The manifestation of UBE2G1 mRNA in each organization of a northern-blot-analysis normal mouse was analyzed using the mouse MTN blot (Clontech make). All RNA extracts from each mouse organization of the wild type obtained in the above-mentioned example 2, a hetero deficit mold, and a gay deficit mold mouse and poly A (+) RNA purification were

performed according to the procedure attached to the reagent.

[0077] That is, ISOGEN (trade name: NIPPON GENE make) which is a modifier was added to a wild type, a hetero deficit mold, and each mouse skeletal muscle of a gay deficit mold mouse, all RNA was extracted, and poly A (+) RNA was refined using oligo tex-DT30 (oligotex-DT30: TAKARA SHUZO CO., LTD. make). Electrophoresis of this poly A (+) RNA3microg was carried out by the formaldehyde method, and it imprinted to the high bond N+ (Hybond N+) nylon film (the Amersham Pharmacia Biotech K.K. make). The protein coding region of mouse UBE2G1cDNA obtained in the example 1 was used for the probe (array number 2). After hybridization, after performing washing for 15 minutes twice in 65 degrees C, 0.1xSSC, and 0.1%SDS solution, the autoradiography which performs exposure to an X-ray film (the product made from KODAKKU: Kodak) at -80 degrees C for 6.5 hours was performed.

[0078] Consequently, in addition to skeletal muscle, mouse UBE2G1mRNA was strongly discovered also in the organization of a liver, a kidney, an alignment, etc. (drawing 4). Moreover, the manifestation of mRNA in UBE2G1 genetic-defect mouse skeletal muscle was accepted also in any of a wild type, a hetero deficit mold, and a gay deficit mold (drawing 5). However, it was possible that it is missing since the 2nd exon part of UBE2G1 gene is permuted by the neomycin resistance gene expression unit, and the reading frame to amino acid shifts as the result, and UBE2G1 normal protein is not produced (drawing 1 , 6). In the Western blot analysis using the anti-UBE2G1 peptide antibody of the example 7 which this thing mentions later, since UBE2G1 protein was not detected in the liver homogenate of a gay deficit mold mouse, it was supported that this gene is destroyed.

[0079] Example 5 A pET21a vector (novagen company make: Novagen) is used for the preparation Homo sapiens UBE2G1 recombination protein (hUBE2G1) of UBE2G1 recombination protein. It was made discovered using a competent cell (Escherichia coli) (Epicurian Coli (trademark) BL21-Codon PlusTM-(DE3) RIL Competent Cells (Stratagene make)).

[0080] The mouse UBE2G1 recombination protein (mUBE2G1-His) which attached six histidine residue (His tag) to the C terminal was discovered in the above-mentioned Escherichia coli cell using pET23 vector (novagen company make).

[0081] Purification the Escherichia coli which discovered mUBE2G1-His 10% cane sugar, With 20mM(s) and the tris-hydrochloric-acid buffer solution (pH7.5) containing 1mMDTT and a protease inhibitor cocktail (sigma company make: Sigma), after ize [HOMOJIE], The obtained supernatant liquid which carried out ultracentrifuge (for 100,000 x g and 30 minutes, 4 degrees C) was applied to the nickel-NTA super flow (Super flow : product made from

QIAGEN), and it carried out by making it eluted with the buffer solution containing an imidazole. The target recombination protein (mouse UBE2G1-His recombination protein) was obtained by the 50 – 100mM imidazole fraction in this way (drawing 7).

[0082] Example 6 The mUBE2G1-His solution which are production of an anti-UBE2G1 peptide antibody and the affinity purification above, and was made and refined was applied to PD-10 column (the Amersham Pharmacia Biotech K.K. make), by making it eluted with 50mM phosphate buffer solution (pH7.0) containing 0.15M NaCl, buffer exchange was performed and the fixed column was produced. 1mM The PD-10 above-mentioned eluate was added to NHS-activation sepharose 4 first FUROU (the Amersham Pharmacia Biotech K.K. make) washed with the hydrochloric acid, and it was made to react for 30 minutes at a room temperature. Subsequently, 0.5M The tris-hydrochloric-acid buffer solution (pH8.0) washed enough, and it put at 4 degrees C into this buffer solution. 2 hours after and 0.5M The acetic-acid buffer solution (pH4.0) and 0.5M After the tris-hydrochloric-acid buffer solution (pH8.0) washed by a unit of 6 times by turns, it washed enough by phosphoric-acid buffer physiological-salt-solution (-) (D-PBS) (NISSUI PHARMACEUTICAL CO., LTD. make) of Dulbecco of an amount, and saved at 4 degrees C till the activity.

[0083] The peptide (SMLADPNGDSPANVD, array number 8) with which UBE2G1 gene consists of 15 amino acid equivalent to the 126-140th by the side of the C terminal of the protein which carries out a code for antibody production is compounded, and it is a keyhole rim pet hemocyanin (Keyhole Limpet Hemocyanin: (KLH) (product made from Pierce) was combined, and it considered as the antigen.) as carrier protein.

[0084] The immune animal was medicated with the antigen of 250microper one per time g at hypodermically or muscles using two white reg phones (Metz, 83 ages in day). Immunity was performed a total of 4 times every two weeks. It collected blood after [of the 4th immunity] one week, the blood serum was prepared, and it considered as antiserum.

[0085] Affinity purification of an antibody was performed as follows. The mUBE2G1-His fixed column was equilibrated by D-PBS after washing with the 0.1mM glycine-hydrochloric-acid buffer solution (pH2.3). The antiserum diluted with D-PBS to 1/10 was applied to this column. The fraction eluted without adsorbing was applied again and a column, the 0.1M glycine-hydrochloric-acid buffer solution (pH2.3) of this capacity, and the antibody to which it subsequently stuck by D-PBS of the one half of the capacity of column were made enough eluted after washing in D-PBS of an amount. 1M [little in that case, in order to neutralize pH in a fraction tube] The tris-hydrochloric acid (pH8.0) is put in and it mixed promptly after elution. Western blot analysis which used mUBE2G1-His as the antigen about

each fraction was performed, fractions with high antibody titer were collected, and it saved at -20 degrees C as a glycerol solution 50% after concentration by Centricon -100 (Centricon-100: Millipore Corp. make).

[0086] Example 7 In order to consider a UBE2G1 protein manifestation with the mouse by which the Western blot analysis gene disruption was carried out, After extracting the liver of a wild type, a hetero deficit mold, and a gay deficit mold mouse, make it freeze in liquid nitrogen promptly, and it crushes with the Cryo press (Cryopress). 20mM(s) containing 1mM EDTA, 1mM DTT, 20% cane sugar, and the protease inhibitor cocktail diluted 50 times Fractionation of the supernatant liquid obtained by carrying out ultracentrifuge after ize [HOMOJIE] with the tris-hydrochloric-acid (pH7.5) buffer solution was carried out by DEAE-sepharose CL-4B. Since it was shown from the experiment using recombination protein that UBE2G1 is eluted in a 0.1M NaCl fraction, these fractions of mouse liver were collected and Western blot analysis was performed using the above-mentioned antibody (drawing 8 , drawing 9).

[0087] Although the manifestation of UBE2G1mRNA was accepted with the UBE2G1 gay deficit mold mouse, since the 2nd exon part is permuted by the neomycin resistance gene expression unit so that it may mention above in an example 4, deletion is carried out, the reading frame to amino acid shifted as the result, and it was expected that UBE2G1 normal protein is not produced. As a result of the experiment concerned, as shown in drawing 9 , it was shown that UBE2G1 protein is not detected in the liver homogenate of a gay deficit mold mouse, but this gene is destroyed as expected in the Western blot analysis using an anti-UBE2G1 peptide antibody (drawing 9).

[0088] Example 8 Under anesthesia, an incision in the abdomen and an after main artery are cut open, and bleeding to death of each mouse of a pathology histological retrieval wild type, a hetero deficit mold, and a gay deficit mold was carried out, and after extracting an organ, neutral buffered formalin was used and it fixed for three days in the cool place. After alcoholic dehydration, embedding was carried out to paraffin and the intercept with a thickness of 3 micrometers was produced. Special staining procedures, such as an azan Mallory stain and silvering dyeing, were performed if needed besides a hematoxylin and eosin stain.

[0089] As a result of autopsying an age mouse pathologically for nine to 11 months, it applied to adventitia from the cardiac media by nine examples among 21 gay deficit mold mice, and fibrosis was accepted (drawing 10 , drawing 11). However, in the fiber blow hole, the inflammatory cell as which fibroblast is regarded was not accepted at all. Moreover, in the age mouse, the ratio of the cardiac weight to weight was larger than the genotype mouse of others [a gay deficit mold mouse] in the same month. Furthermore by the mesangium of a UBE2G1 genetic-defect mouse, the increment in a

mesangium substrate was accepted (drawing 12 , drawing 13).

[0090] Example 9 As shown below, scoring by two observers' blank test performed assessment of the assessment mesangium lesion of a mesangium lesion. That is, the glomerulus which exists in the renal-cortex surface section of the same intercept was selected at random, and according to the condition of a mesangium field, it classified into the three-stage and mark-ized (0 (drawing 14):0 Type, 1 (drawing 15):1 Type, 2 (drawing 16):2 Type(s)).

[0091] 100 glomeruli per one individual were observed, those mark were totaled, and it considered as the score of the individual. Male Metz was evaluated about three groups (each seven to group 10 individual) of a wild type, a hetero deficit mold, and a gay deficit mold. the obtained data -- a randomized blocks method model (factor: -- genotype and an observer --) Genotype x Analysis-of-variance [Huynh by the observer, H and Feldt and L.S.:(1976) Estimation of the box correction for degrees of freedom for sample data in randomized block and split-plot designs.; J.Educational Statistics, 1, and 69-82 are performed. It is related with a difference between groups. ** turkey (Tukey) assay (Tukey, J.W.; (1949) Comparing individual means in the analysis ofvariance.; Biometrics 6, 90-114) was performed. Level of significance of assay in this case was made into 5%, and was authorized with SAS software (made in SAS Japan, R6.12).

[0092] When the mesangium score of three groups of a wild type (+/+), a hetero deficit mold (+/-), and a gay deficit mold (-/-) was compared, with the gay deficit mold mouse (-/-), the high score was obtained more nearly intentionally than a wild type mouse (+/+) and a hetero deficit mold mouse (+/-) (drawing 17), and it was shown that the mesangium field is increasing.

[0093] Example 10 Under anesthesia, an incision in the abdomen and a bottom main artery are cut open, and bleeding to death of the immunohistochemistry-analysis mouse was carried out, and after extracting an organ, neutral buffered formalin was used and it fixed in the cool place overnight. After alcoholic dehydration, embedding was carried out to paraffin and the intercept with a thickness of 3 micrometers was produced.

[0094] As an antibody, an FITC indicator anti-mouse IgG antibody (20 time dilution, sigma company make), An anti-collagen type IV antibody (500 time dilution, product made from LSL), an anti-collagen type I antibody (time [100] dilution, the product made from biotechnology JINESHISU: Biogenesis), An anti-tee necine antibody (1000 time dilution, product made from CHEMICON International:CHEMICON International), An anti-DESUMISHIN antibody (time [300] dilution, made in ICN fur MASHU Tikal: ICN Pharmaceutical), Or alpha[anti-]-smooth-muscle actin antibody: Alpha-smooth muscle actin (dilution 25 times) The product made from DAKO: Use DAKO and, in the case of an FITC indicator anti-mouse IgG antibody, in the case of other

antibodies, it is VECTASTAIN R with a fluorescent antibody technique. It detected in the enzyme-labeled antibody technique using an ABC kit (product made from VECTOR).

[0095] Consequently, there is no deposition of an immunoglobulin in the glomerulus of a UBE2G1 gene deficient mouse, and it was thought that the inflammation which made the deposition of an immune complex the mechanism did not participate in this lesion. Since the manifestation of alpha-smooth muscle actin was increasing by the glomerulus to which extracellular matrices, such as the collagen type's IV and a basement membrane, increasing furthermore and a lesion reached an advanced stage, it is heard that a mesangial cell is activated by a certain mechanism and production of an extracellular matrix is accelerating by it.

[0096]

[Layout Table]

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[Translation done.]

* NOTICES *

JPO and NCIP1 are not responsible for any damages caused by the use of this translation.

- 1.This document has been translated by computer. So the translation may not reflect the original precisely.
- 2.**** shows the word which can not be translated.
- 3.In the drawings, any words are not translated.

DESCRIPTION OF DRAWINGS

[Brief Description of the Drawings]

[Drawing 1] It is structural drawing of the gene of UBE2G1 wild type (intact) and a deficit mold (mutant).

[Drawing 2] Drawing A is structural drawing of UBE2G1 wild type (Intact Allele), the deficit mold (Mutant Allele) by homologous recombination, and a targeting vector (Targeting vector). Moreover, drawing B is drawing showing the result of having judged the genotype (a wild type (+/+), a hetero deficit mold (+/-), gay deficit mold (-/-)) of a UBE2G1 genetic-defect mouse in the example 3.

[Drawing 3] It is drawing which saw change of the weight of a wild type mouse (+/+), a UBE2G1 gene hetero deficit mold mouse (+/-), and a UBE2G1 gene gay deficit mold mouse (-/-) with time. Drawing A is a female mouse and drawing B is as a result of a male mouse.

[Drawing 4] Manifestation drawing of UBE2G1mRNA in the various organizations of a mouse is shown.

[Drawing 5] It is drawing showing the result of having seen the manifestation of mRNA in UBE2G1 genetic-defect mouse skeletal muscle about the wild type mouse (+/+), the hetero deficit mold mouse (+/-), and the gay deficit mold mouse (-/-), respectively.

[Drawing 6] It is drawing which compared with the normal amino acid sequence the variation amino acid sequence produced by the UBE2G1 gene targeting.

[Drawing 7] It is the drawing in which the zymogram which shows extent of purification of mouse UBE2G1-His recombination protein is shown.

[Drawing 8] It is the drawing in which the detection result of UBE2G1 of the mouse liver DEAE fraction by the anti-UBE2G1 peptide antibody is shown.

[Drawing 9] It is drawing showing the existence of a manifestation of UBE2G1 molecule about UBE2G1 wild type (wild), a hetero deficit mold (hetero), and a gay deficit mold (KO) mouse.

[Drawing 10] It is the organization chart of the heart cross section of a UBE2G1 gay deficit mold mouse.

[Drawing 11] It is the amplification organization chart of the heart cross section of a UBE2G1 gay deficit mold mouse.

[Drawing 12] It is the strong amplification organization chart of the mesangium cross section of a UBE2G1 wild-type mouse (nine-month age).

[Drawing 13] It is the strong amplification organization chart of the mesangium cross section of a UBE2G1 gay deficit greensand-mold mouse (nine-month age).

[Drawing 14] It is the organization chart of the mesangium cross section of a mouse showing normal mesangium (Type0: zero score).

[Drawing 15] It is the organization chart of the mesangium cross section of a mouse showing growth of the partial mesangium substrate of mesangium (Type1: one score).

[Drawing 16] It is the organization chart of the mesangium cross section of a mouse showing growth of the overall mesangium substrate of mesangium (Type2: two scores).

[Drawing 17] It is drawing showing the result of having compared the score of a mesangium lesion, about UBE2G1 wild type (+/+), a hetero deficit mold (+/-), and a gay deficit mold (-/-) mouse. Drawing A is a female mouse and drawing B is as a result of a male mouse.

[Translation done.]